Remarks

Claims 1-33 are pending. No claims are added or cancelled. Claim 27 has been amended to include G8-PAMAM dendrimer conjugates. Support for this amendment can be found in the originally filed specification and claims, including Examples 2 and 3. No new matter is added by this amendment. Reconsideration of the outstanding rejections is requested in view of the following remarks.

I. Status of January 13, 2005, Preliminary Amendment

Applicants filed a Preliminary Amendment on January 13, 2005, making certain corrections to the specification. Applicants would appreciate it if the Office would confirm that this Preliminary Amendment has been considered and entered.

II. Claim Rejections – 35 U.S.C. § 103(a)

The Office action states that claims 1-33 are rejected under 35 U.S.C. § 103(a) as being obvious over Suga et al. (*Acta Radiologica* **2003**, 44, 35-42) ("Suga") in view of U.S. Patent No. 7,261,875 to Li et al. ("Li") and U.S. Patent No. 6,471,968 to Baker, Jr. et al. ("Baker"). It appears that this combination of references further includes U.S. Patent No. 7,081,452 to Brechbiel et al. ("Brechbiel").

Suga is asserted to disclose using MR lymphography to analyze lymphatic system drainage using gadopentetate dimeglumine, Gd-DTPA-PE-POE, and SPIO particles. The Office action admits that Suga contains no disclosure of using PAMAM contrast agents in such methods. Li is asserted to disclose the use of Gd-DTPA-PAMAM and -DAB dendrimer conjugates as MRI contrast agents. The Office action states that Li discloses the use of such contrast agents to treat cancer of the lymph node. Brechbiel is asserted to disclose the use of 1B4M-DTPA chelates, while Baker is asserted to disclose the use of fluorescent tagged Gd-PAMAM dendrimer conjugates.

As an initial matter, Applicants respectfully assert that the combination of references cited in the Office action does not provide all features of Applicants' claims. For example, the Office action states that Li provides PAMAM and DAB dendrimer conjugates. However, it appears that Li actually provides polymeric materials formed by polymerizing N-carboxyanhydride monomers using a dendrimer, such as a PAMAM or DAB dendrimer, as an

initiator. *See* col. 4, ll. 24-46; col. 10, l. 48-col. 11, l. 19. Accordingly, it appears that each arm of the dendrimer contains a number of poly(amino acids) as terminal functional groups. As noted in the "Summary," the therapeutic and diagnostic methods of Li appear to include attaching a therapeutic agent or diagnostic agent to the poly(amino acid) chains of the modified dendrimer, not to the dendrimer itself. Col. 4, l. 60 – col. 5, l. 8. Although this Response discusses a number of other differences arising from Li's use of modified dendrimers, the fact that Li does not use gadolinium chelates of PAMAM or DAB dendrimers by itself establishes that the asserted combination of references does not teach or suggest all aspects of Applicants' claims. As discussed below, Li also teaches away from using larger dendrimers, such as those claimed by Applicants, as the bases for its modified dendrimers. For this reason alone, Applicants respectfully request that the § 103(a) rejections of the pending claims be withdrawn.

The Office action asserts that, at the time of Applicants' invention, it would have been obvious to try and use the PAMAM poly(amino acid) dendrimer conjugates of Li in the method of Suga, further replacing the chelate with the 1B4M conjugate of Brechbiel. Using dendrimers to increase the water solubility and diagnostic agent loading of therapeutic agents is asserted to provide the motivation for the substitution of PAMAM or DAB dendrimers in the methods of Suga. Applicants respectfully disagree.

According to the recent revisions to MPEP § 2143, a *prima facie* case of obviousness based on an "obvious to try" rationale requires the Office to establish:

> (1) a finding that at the time of the invention, there had been a *recognized problem* or need in the art, which may include a design need or market pressure to solve a problem;
>
> (2) a finding that there had been a *finite number of identified, predictable potential solutions* to the recognized need or problem;
>
> (3) a finding that one of ordinary skill in the art could have pursued the known potential solutions with a *reasonable expectation of success*; and
>
> (4) whatever additional findings based on the *Graham* factual inquiries may be necessary, in view of the facts of the case under consideration, to explain a conclusion of obviousness.

MPEP § 2143(E) (emphasis added). An analysis of these factors reveals that the Office action fails to establish multiple factors required for a *prima facie* case of obviousness.

The Office Action Does Not Identify a Recognized Problem in Suga

Regarding the first factor, the Office action states that one of ordinary skill in the art would have been motivated to use the PAMAM poly(amino acid) dendrimer conjugates of Li in place of the Gadolinium chelates of Suga because of the possibility of increasing the loading of active agent and of providing complexes with improved water solubility. However, the Office action does not appear to provide any support for the proposition that these factors were a "problem" in the methods of Suga or were recognized as such. For example, the "background" section of Li does not appear to discuss solubility or loading problems with Gd-chelates in imaging applications, rather it focuses on the need to enhance "targeting" of therapeutic agents so that less agent can be used with fewer side effects resulting from contact with untargeted areas. Accordingly, the problem identified by Li is targeting, not solubility or loading. Col. 1, l. 30-col. 2, l. 52. Moreover, the disclosure of Li teaches away from increasing the loading of an untargeted agent, as that would be expected to further exacerbate the side effects or toxicity to untargeted areas.

Unless a recognized problem was present in the Suga methods, Applicants respectfully assert that there would have been no motivation to use the dendrimer conjugates of Li in Suga, even if they did provide the benefits asserted in the Office action. Because no recognized problem has been identified (and the problems identified by Li teach away from Applicants' invention), Applicants respectfully assert that the Office action fails to establish a *prima facie* case of obviousness. Accordingly, Applicants respectfully request that the § 103(a) rejections of the pending claims be withdrawn.

The Cited Combination Does Not Provide a Finite Number of Predictable Solutions

Regarding the second factor, Applicants respectfully assert that the Office action does not establish that there were only a finite number of identified, predictable solutions, even if a valid, recognized problem had been presented which would satisfy the first factor. Assuming one of ordinary skill in the art was aware of the Suga reference and was motivated to try to increase the solubility or loading of diagnostic agent, where would they look to find candidates for suitable agents? Even assuming they would have looked to polymeric agents, what motivation would they have had to select dendritic agents, much less the specific agents disclosed and claimed by Applicants? Given the number of possible linear and dendritic polymers and chelating agents, Applicants respectfully assert that there would not be a finite number of identified potential solutions to the problem. Furthermore, as previously noted, the disclosure of Li appears to

suggest modifying dendrimers with poly(amino acids), vastly increasing the number of potential agents. Applicants assert that, if anything, Li would suggest adding targeting moieties to the agents of Suga, not selecting the untargeted dendrimer conjugates of Applicants' claims.

Li further teaches away from using unmodified dendrimers by suggesting that their properties are not suitable for diagnostic or therapeutic applications without modification. For example, Li states:

> However, unlike dendrimers, the synthesis of these carriers does not require tedious, multistep procedures, and they are very inexpensive to prepare as compared to dendrimers. Additionally, these carriers may be biodegradable and have heterofunctional groups for drug attachment and for coupling of targeting moieties.

Col. 7, l. 66-col. 8, l. 4. The properties of the Li compounds are quite different than those of unmodified PAMAM dendrimer conjugates. For example, Table 1 of Li notes that G2-PAMAM with an ethylenediamine core has a molecular weight of 3256. The poly(amino acid) dendrimer using a ethylenediamine core prepared according to Li has a molecular weight of 96460, which is over 29 times heavier than the PAMAM dendrimer. As explained in more detail below, differences in size, shape, core, weight, charge, and other properties would be expected to affect the pharmacological properties of the dendrimer conjugates. Thus, the poly(amino acid) dendrimers of Li would further increase the unpredictability of potential solutions, as well as vastly increasing the number of potential agents.

Applicants also note that the pending claims are directed to dendrimer conjugates having DAB and PAMAM dendrimers of generation 4-8. There is no teaching or suggestion in any of the cited references, or combination thereof, to use these agents for imaging the lymphatic system. In fact, Li teaches away from using dendrimer conjugates having a dendrimer larger than generation 2. Although Table 1 of Li generically lists PAMAM dendrimers of generations 0-7, the Examples of Li do not employ any dendrimer larger than generation 2 (for example, PAMAM-PG_{16} of Example 1 has 16 terminal amino groups, making it a generation 2 dendrimer). In addition, Li presents no data for even the PAMAM poly(amino acid) dendrimer conjugates in actual use as imaging agents, for either *in vivo* or *in vitro* applications, much less any data indicating that any dendrimer conjugate could be used to image the lymphatic system. Through its statements regarding the difficulty of synthesizing larger dendrimers, and their inferior

functionalization characteristics, Li explicitly teaches away from using larger PAMAM or DAB dendrimers.

In addition to the vast number of potential agents, and lack of guidance to select those claimed by Applicants (and in fact Li's teaching away from such agents), dendritic agents, in particular, are known to have unpredictable pharmacological properties. As explained further below, this unpredictability would further reduce any ability one of ordinary skill in the art conceivably had to *a priori* select particular agents that would be suitable for a particular application. For the reasons set forth above, Applicants respectfully request that the § 103(a) rejections of the pending claims be withdrawn.

The Pharmacological Properties of Dendrimer Conjugates Are Unpredictable and Would Not Provide a Reasonable Expectation of Success

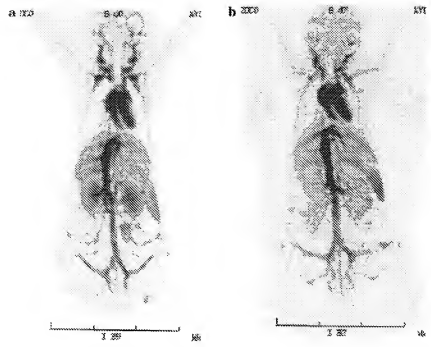
Regarding the third factor, Applicants assert that, prior to the blueprint provided by Applicants' disclosure, those of ordinary skill in the art would have had no reasonable expectation of successfully using the claimed dendrimer conjugates to image the lymphatic system. The unpredictable pharmacological nature of such agents was well known at the time of Applicants' invention. According to MPEP § 2143.02 "at least some degree of predictability is required" in order to support a *prima facie* case of obviousness.

It has been recognized that the pharmacological properties of even structurally similar dendrimer conjugates are unpredictable. For example, Kobayashi et al. noted that two generation-6 PAMAM dendrimer conjugates having similar size, but dissimilar molecular weights and cores, exhibited different pharmacokinetic properties. *Bioconjugate Chem.* **2001,** 12, 100-107 ("Kobayashi I"); *see* also U.S. Patent Publication US 2005/0019267 at ¶ 14.

> When the 1B4M was conjugated with all amino-groups on the surface of the molecule, the chemical properties of both molecules were then expected to remain similar. However, not only G6E-$(1B4M\text{-}Gd)_{256}$ versus G6A-$(1B4M\text{-}Gd)_{192}$, but also G6E-$(1B4M\text{-}Gd)_2$ versus G6A-$(1B4M\text{-}Gd)_2$, showed different pharmacokinetics in vivo, especially in the kidney (glomerular filtration).

Kobayashi I at 105.

In Kobayashi I, images obtained using the different agents demonstrated vastly different imaging capabilities. As shown in the figures below, after administration to a mouse, the G6A agent clearly distinguished kidneys from surrounding organs. The images using the G6E agent were much less clear.



Kobayashi I at 105. Similarly, another publication by Kobayashi et al. (*Mol Imaging* **2003**, *2*, 1-10) ("Kobayashi II") teaches that the pharmacokinetic properties of dendrimer conjugates are affected by their size, with G2-G6 agents being rapidly excreted by the kidney, while larger agents, such as G9 and G10, were "quickly taken up and trapped in the reticuloendothelial system (liver and spleen) resulting in rapid clearance from the circulation." P. 5 (internal citations omitted). Yet another publication by Kobayashi et al. (*Cancer Research* **2003**, *63*, 271-276) ("Kobayashi III") notes that the pharmacokinetic properties of dendrimer-conjugates can vary greatly depending on the size of the conjugate:

> The PAMAM dendrimer-core based macromolecular MRI contrast agents could visualize blood vessels (larger generations) or kidney tubules (smaller generations). In the molecular weight range studied previously, the larger molecules, PANAM-generation-7 [sic] and PAMAM-G8 remained in the circulation longer because of less excretion from the kidney, resulting in visualization of finer vessels.

P. 275 (internal citations omitted).

As demonstrated by Kobayashi I-III, at the time of the invention, those of ordinary skill in the art would have known that even structurally similar agents have very different imaging qualities. One of ordinary skill in the art would have had no expectation of what, if any,

physiologically useful imaging properties might exist for a given agent until such agent had been prepared and tested *in vivo*.

Even among agents suitable for imaging some portion of the lymphatic system, particular agents have different abilities to image different portions of the lymphatic system. For example, agents suitable for imaging the lymphatic vessels may be unsuitable for imaging the lymph nodes. Kobayashi et al., in another publication (*J. Natl. Cancer Inst.* **2004**, *96*, 703-708) ("Kobayashi IV"), note that the pharmacological properties of dendrimer-conjugates can vary greatly in their ability to image the lymphatic system depending on the nature of the conjugate:

> The ideal imaging agent should be small enough to rapidly enter into lymphatic vessels and flow with the lymph fluid yet large enough to stay within the lymphatic system and not leak into capillary vessels. Previous reports found that lymphangiographic contrasts agents must be at least 4 nm in diameter to be retained efficiently within the lymphatic system. Molecules smaller than 4 nm in diameter penetrate capillary membranes and diffuse into the circulatory system, resulting in poor signal-to-background ratios. Larger molecules, by contrast, diffuse slowly from the interstitial space and likely accumulate more slowly in the sentinel nodes, providing a longer imaging window for visualizing these nodes. The G8 agent (13 nm in diameter) used in our previous report for deep lymphatic imaging studies is too large for rapid uptake by lymphatic vessels (data not shown), whereas the G6 contrast agent (9 nm in diameter) used in this study is large enough to be retained in the lymphatic system but not so large that it cannot be taken up efficiently.

P. 707. These findings are consistent with Applicants' specification, which teaches that "The axillary lymph node-to-liver ratio obtained with PAMAM-G4 was significantly higher than that acquired with PAMAM-G8, DAB G-5, or Gadomer-17 at all time points examined ($P<0.01$)." P. 34, ll. 23-25. The specification also states that:

> [E]ach of the dendrimer-based contrast agents exhibited distinct characteristics, which may be exploited for different purposes in clinical applications. For example, PAMAM-G8 appears to be better suited for imaging of lymphatic vessels and diverse other components of the lymphatic system, whereas DAB-G5 may be better suited for imaging of lymph nodes. PAMAM-G4 appears to be particularly suited for visualization of abdominal lymph nodes adjacent to the liver based on its high lymph node to liver signal intensity.

P. 37, ll. 18-24.

Hence it would be unpredictable that randomly selecting a linear or dendritic polymer would provide an imaging agent capable of imaging the lymphatic system in the claimed manner. In fact, for the reasons stated above, those of ordinary skill in the art would have been

dissuaded from using higher generation PAMAM or DAB dendrimers as imaging agent given their stated synthetic difficulty, inferior functionalization properties, potential toxicity, and indiscriminate (non-targeted) distribution. The cited references do not provide the motivation to combine alleged in the Office action and, if anything, teach against the combination.

In any event, a *prima facie* case of obviousness cannot be supported by an allegation that random parts of different references (such as Li and Suga) could be fortuitously combined to arrive at the claimed invention. Such a rejection uses hindsight reconstruction of the claimed imaging agent in view of the applicants' own disclosure, which is not permitted. See MPEP 2142. Instead, the asserted combination of references must identify a recognized problem that would lead to the combination, a finite number of predictable potential solutions, and a reasonable expectation of success. For the reasons set forth above, Applicants respectfully assert that none of these factors are established in the Office action. Applicants request that the § 103(a) rejections of claims 1-33 be withdrawn.

Applicants' Agents Demonstrate Unexpected Superior Results

Even if a *prima facie* case of obviousness had been established, Applicants' claims would nevertheless be patentable in view of the unexpected superior results demonstrated in Applicants' specification. According to MPEP § 2145, "Usually, a showing of unexpected results is sufficient to overcome a *prima facie* case of obviousness." As discussed above, the pharmacological properties of dendrimer conjugate imaging agents were known to be unpredictable at the time of Applicants' invention. The dendrimer conjugates exhibited superior imaging properties, even as compared with other dendrimer conjugates.

Applicants' specification provides a number of examples demonstrating the superior qualities of the claimed dendrimer conjugates in imaging the lymphatic system. Among the agents used for comparative purposes, Applicants present data for Gadomer-17, a polylysine dendrimer containing 24 gadolinium ions bound by a DO3A chelating agent marketed by Schering AG (Berlin, Germany). Applicants' specification notes that:

> The lymph nodes (particularly those about 2/3 of the way up the body of the mice) appear much brighter and well-defined in the images obtained using the dendrimer conjugates than in the images obtained with Gadomer-17 and GPDM. As shown in FIG. 8B, the dendrimer conjugates exhibit persistent superior image

contrast and detail of the lymphatic system 45 minutes after injection in comparison to Gadomer-17 and GPDM.

P. 33, l. 24 – p. 34, l. 1. As previously noted above, the claimed dendrimer conjugates also exhibited superior axillary lymph node-to-liver image enhancement compared with Gadomer-17.

Applicants specifically present data for generation-4, -6, and -8 PAMAM and generation-5 DAB dendrimer conjugates. The remaining claimed PAMAM dendrimer conjugates fall within the range of generation 4-8. Accordingly, Applicants assert that the demonstration of unexpected superior results is reasonably commensurate with the scope of the claims. *See* MPEP § 2145 (stating "a showing of unexpected results for…a narrow portion of a claimed range would be sufficient to rebut a *prima facie* case of obviousness if a skilled artisan 'could ascertain a trend in the exemplified data that would allow him to reasonably extend the probative value thereof'."). Additionally, the specific dendrimer conjugates whose unexpected results are demonstrated in the application are specifically claimed in claims 2, 3, 9, 10, 17, 21, 27, and 28.

Accordingly, even if a *prima facie* case of obviousness had been established, Applicants' claims would nevertheless be patentable in view of the demonstrated unexpected superior results in imaging the lymphatic system. For this additional reason, Applicants respectfully request that the § 103(a) rejections of claims 1-33 be withdrawn.

III. Conclusion

Applicants submit that the present application is in condition for allowance. If the Examiner has any questions regarding the application or this response, the Examiner is encouraged to call Applicants' attorney, Ryan A. Heck, at (775) 824-0104.

Respectfully submitted,

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